75.007

Serodiagnosis of tuberculosis using nine *in silico* predicted B-cell epitopes peptides derived from *Mycobacterium tuberculosis* proteins

L. Baassii[1,*], K. Sadki[2], F. Seghrouchni[2], S. Contini[3], W. Cherki[2], N. Nagelkerke[4], A. Benjouad[5], C. Saltini[3], V. Colizzi[3], R. El aouad[2], M. Amicosante[3]

[1] *National Institute of Hygiene- Ministry of Health, Rabat, Morocco*
[2] *National Institute of Hygiene, Rabat, Morocco*
[3] *University of Rome ''Tor Vergata'', Rome, Italy*
[4] *United Arab Emirates University, Al Ain, United Arab Emirates*
[5] *Université Mohamed V, Rabat, Morocco*

Background: Rapid, simple, and new diagnostic tools are needed for the diagnosis of tuberculosis (TB). The aim of this study is to identify a combination of *Mycobacterium tuberculosis* (*Mtb*) peptides useful for the serodiagnosis of active pulmonary tuberculosis (TBp).

Methods: Fifty-seven B-cell epitopes peptides of *Mtb* were selected by *in silico* methods and evaluated by immunoenzymatic assay. Two sample panels were used for evaluation: (i) twenty pulmonary tuberculosis (TBp) patients and ten healthy subjects (HS) from a country with low incidence of TB (Italy) and (ii) forty-seven TBp patients and 26 HS from a country with high incidence of TB (Morocco) and the data were analyzed using logistic regression analysis and Random Forest method.

Results: The best discriminating peptide between TBp patients and HS from the sample of the country with low incidence of TB has been the 23 amino acid peptide of the Rv3878 protein. Thus, the sensitivity and specificity was 65% and 100%, respectively. In contrast, the same peptide showed 47% and 100% as sensitivity and specificity respectively in the country with high incidence of TB. In addition, the best peptides combination was a pool of nine peptides which showed a sensitivity of 70.2% and a specificity of 100% in the country with high incidence of TB.

Conclusion: The present study showed that the 9-peptides pool can be useful in identifying patients with active pulmonary tuberculosis.

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Cerebrospinal and blood nitric oxide in tubercular meningitis

S. Jain*, R. Srinivas, N. Sharma, M. Khullar

PostGraduate Institute of Medical Education and Research, Chandigarh, India

Background: The role of nitric oxide (NO) in central nervous system infections is controversial. Nitrites, the stable end product of NO metabolism as reactive nitrogen intermediates (RNI), an index of NO synthesis, were measured in CSF and blood of patients with tubercular meningitis (TBM).

Methods: 40 patients with TBM and 50 matched controls were recruited for the study. The diagnosis was based on clinical features, CSF features (pleocytosis of more than 10 cells/mm3 with >50% lymphocytes, sugar< 50% of blood sugar, protein > 40mg% and Adenosine deaminase (ADA) > 8U/L)or a positive PCR or culture for Mycobacterium tuberculosis in CSF or a suggestive CT Scan of brain. Controls were patients undergoing elective surgery for nonneurological conditions under spinal anaesthesia.

Results: The clinical profile of the patients (mean age 30.6 ± 12.3 years) included fever in 38 (95%), headache in 36 (88%), seizures in 11 (28%) and focal neurological deficits in 6 (15%) patients. Total leukocyte and relative lymphocyte counts in CSF of patient's were $1650 \pm 157/mm^3$ and $76 \pm 31\%$ respectively. ADA was more than 8U/L in 31(77.5%) and PCR for M. tuberculosis was positive in 10 (19 samples). CT brain showed basal exudates in 13(32.5%), gyral enhancement in 8(20%), infarcts in 12(30%) and hydrocephalus in 19(47.5%) patients. CSF-RNI levels in patients ($16.9 \pm 19.5\ \mu mol/L$) were higher than in controls ($1.3 \pm 1.1\ \mu mol/L$)($p < 0.01$). Serum RNI levels of patients ($53.5 \pm 13.8\ \mu mol/L$) were higher than controls ($3.8 \pm 1.9\ \mu mol/L$)($p < 0.01$). There was no correlation between CSF-RNI and blood-RNI or between CSF-RNI levels and the biochemical or clinical parameters. Two patients died on follow up related to the disease.

Conclusion: CSF and blood NO levels are increased in tubercular meningitis and are not related to clinical outcome suggesting that NO is a non-specific marker of CNS inflammation and can not be incriminated as an agent of tissue damage.

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Combinatorial use of IgG antibodies to secreted mycobacterial proteins to create a screening test for childhood tuberculosis

Z. Araujo[1,*], F. Giampietro[2], M.A. Patarroyo[3], C. Fernandez de Larrea[4]

[1] *Instituto de Biomedicina, Universidad Central de Venezuela, 4043, D.C., Venezuela*
[2] *Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, D.C., Venezuela*
[3] *Fundación Instituto de Inmunología de Colombia, Bogotá, Colombia*
[4] *Servei d'Hematologia. Hospital Clínic de Barcelona, Barcelona, Spain*

Background: To create a rapid, simple and relatively inexpensive screening strategy for childhood tuberculosis (TB) that includes antibody detection assays to improve the accuracy of microscopic examination of sputum for acid-fast bacilli (AFB smear) in Warao indigenous childhood TB given that TB is difficult to diagnose, and invasive procedures cannot be used to select samples in these communities.

Methods: TB diagnosis was established by *Mycobacterium tuberculosis* culture, clinical diagnosis, thorax radiography and smears stained with Ziehl-Neelsen. HIV status was determined by commercial serologic test. Efficacy and diagnostic potential of different secretory antigens of *Mycobacterium tuberculosis* (rESAT-6, (17) Ag85A and (5) ESAT-6 synthetic peptides) in the detection of specific IgG antibody profiles of childhood TB cases were evaluated using ELISA technique

and sensitivity was compared with the gold standards (smear and culture). A total population of 117 children under the age of 15 years old residing in the visited indigenous communities was examined. Secretions of the pharynx and attempts to obtain samples of sputum by expectoration in children older than 10 years old was carried out in all highly suspected pulmonary TB cases. Serum samples were obtained from 39 untreated children patients, and 78 healthy children. ROC curve analysis was used to calculate the sensitivity and specificity of each antigen for antibody detection.

Results: The results revealed no case of HIV-positive TB among Warao children. Bacteriological confirmation had 8.8% sensitivity, Ag85A peptides showed better sensitivity and specificity than ESAT-6 peptides; anti-29880 peptide test was found to be showing highest sensitivity of 100.0% (Negative Predictive Value, NPV = 100) but a low specificity of 20.8%. Two tests were highly specifics, anti-11003 had 97.4% specificity (Positive predictive Value, PPV = 85.7) and 32.4% sensitivity and anti-10999 had 96.2% specificity (PPV = 86.4) and 48.7% sensitivity. Compared to bacteriological tests, sensitivity of a combination that included a two-antigen ELISA (29880 and 11003 synthetic peptides) was significantly higher, $p < 0.0001$.

Conclusion: Our results demonstrate that the potential of combinatorial use of antibodies directed at different epitopes of Ag85A protein could provide a screening strategy for developing a multi-antigen ELISA, which allows an increase in the diagnostic accuracy of pulmonary TB in Warao childhood population.

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Specificities of the APTIMA Combo 2 and ProbeTec for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in oropharyngeal and rectal specimens from MSM

J. Schachter[1,*], J. Moncada[1], A. Roger[1], L. Rauch[2], S. Liska[2], C. Shayevich[2], J.D. Klausner[2]

[1] *University of California, San Francisco, San Francisco, CA, USA*
[2] *San Francisco Department of Public Health, San Francisco, CA, USA*

Background: Nucleic acid amplification tests (NAATs) are not FDA-cleared for diagnostic testing of chlamydial (CT) or gonococcal (GC) infection in extragenital sites. In men who have sex with men (MSM), CT and GC infections of the oropharynx or rectum may be common. There have been some concerns about false-positive NAAT results from these sites. We evaluated the APTIMA Combo2 (AC2, Gen-Probe Inc.) and ProbeTec (SDA, Becton Dickinson, Co.) performance on these specimens. Here we present the specificity results.

Methods: Oropharyngeal and rectal swabs were obtained from MSM in an STD clinic and SDA and AC2 were performed on all specimens. NAAT positive specimens were retested by AC2, SDA and by APTIMA CT Assay (ACT), or APTIMA GC Assay (AGC) which target rRNA sequences different from AC2.

Results: We tested 1110 MSM. AC2 had more positive results than SDA for both organisms at both sites. The number of positive results, and the percentage confirmed are presented in the table. Only 11 oropharyngeal CT infections were detected, but the number of positive GC specimens and rectal CT specimens was larger allowing more confidence in the reliability of the confirmation data. Using a combination of all 3 tests confirmed >90% of positive samples, resulting in high specificities (>99.6%) for both AC2 and SDA.

	SDA				AC2			
	CT		GC		CT		GC	
(N)	Phx (6)	Rec (43)	Phx (69)	Rec (70)	Phx (11)	Rec (67)	Phx (81)	Rec (85)
Confirmed by (%):								
AC2 or SDA	100*	93	83	99	55	60	70	81
ACT or AGC	100	91	98**	99†	82	93	91	94
Repeat test	100	98	91	97	82	93	91	94
All 3	100	100	94	99	91	94	98	98

% confirmed, **only 57 tested by AGC, † 1 not tested by AGC.

Conclusion: In our MSM population, positive results obtained with AC2 and SDA are reliable, with PPVs >90%. The CT and GC NAAT positives were confirmed to a high degree. As expected, repeat testing, or using the ACT or AGC test, confirm more of the AC2 positive results than did the less sensitive SDA.

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Reverse transcritpase multiplex PCR for detection of viral agents in central nervous system infections

V. Gopalakrishnan[1,*], H. Amar Singh[2], T. Sadanandan[1], G. Perkash Singh[1]

[1] *UniKL RCMP, Ipoh, Malaysia*
[2] *Consultant Paediatrician and Head of Paediatric Department, Raja Permaisuri Bainun Hospital, iPOH, Malaysia*

Background: Viral infections of the central nervous system (CNS) may result in clinical syndromes like aseptic meningitis, encephalitis, and myelitis. These are often difficult to diagnose using conventional laboratory methods, such as viral culture and serology, because they are time consuming and unsatisfactory. Therefore rapid techniques should be employed to detect the etiologic agent. The study was aimed to standardize reverse transcriptase (RT) multiplex PCR aimed to detect viral etiology in CNS infections.

Methods: An RT multiplex PCR designed to detect, viral etiologies, enterovirus, herpes simplex and varicella zoster viruses in CNS infections has been standardized. Three sets of primers were been employed for their detection. Amplification of target sequences was qualitatively analyzed by looking for the presence or absence of amplicons on agarose gel. The RT multiplex PCR was standardized. Sensitivity of the PCR has been ascertained.

Results: Analysis of cerebrospinal fluid samples from pediatric patients is underway. *Conclusion:* The RT multiplex PCR standardized can be employed to detect CNS infections caused by herpes, varicella and entero viruses.

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